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Mutations at the Ataxia-Telangiectasia Locus and Clinical Phenotypes of A–T Patients

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Mutations at the ataxia-telangiectasia (A–T) locus on chromosome band 11q22 cause a distinctive autosomal recessive syndrome in homozygotes and predispose heterozygotes to cancer, ischemic heart disease, and early mortality. PCR amplification from genomic DNA and automated sequencing of the entire coding region (66 exons) and splice junctions detected 77 mutations (85%) in 90 A–T chromosomes. Heteroduplex analysis detected another 42 mutations at the A–T locus. Out of a total of 71 unique mutations, 50 were found only in a single family, and 51 had not been reported previously. Most (58/71, 82%) mutations were frameshift and nonsense mutations that are predicted to cause truncation of the A–T protein; the less common mutation types were missense (9/71, 13%), splicing (3/71, 4%) and one in-frame deletion, 2546 3 (1/71, 1%). The mean survival and height distribution of 134 A–T patients correlated significantly with the specific mutations present in the patients. Patients homozygous for a single truncating mutation, typically near the N-terminal end of the gene, or heterozygous for the in-frame deletion 2546 3, were shorter and had significantly shorter survival than those heterozygous for a splice site or missense mutation, or heterozygous for two truncating mutations. Alterations of the length or amino acid composition of the A–T gene product affect the A–T clinical phenotype in different ways. Mutation analysis at the A–T locus may help estimate the prognosis of A–T patients. Am. J. Med. Genet. 92:170–177, 2000.

KEY WORDS: ataxia-telangiectasia; mutation; survival; phenotype; genotype; DNA sequencing

INTRODUCTION

Mutations at the 150-Kb ataxia-telangiectasia (A–T) locus cause or predispose to disease. Individuals with such mutations on both chromosomes 11 have a distinctive autosomal recessive disorder characterized by progressive neurological degeneration, immunodeficiency, high cancer incidence, and extreme sensitivity to ionizing radiation. Patients with this disorder are referred to as A–T "homozygotes" even when they are more accurately described as compound heterozygotes because each of their chromosomes 11 carries a different mutation at the A–T locus. Some patients, typically from a consanguineous marriage, are true homozygotes.

A–T heterozygotes, who carry a mutation at this locus on only one chromosome 11, have been shown to have an elevated risk of cancer, especially female breast cancer, of ischemic heart disease, and of early mortality [Swift et al., 1991; Athma et al., 1996].

Mutation detection at this locus is arduous because the A–T gene spans 150 kilobases, with an open reading frame of 9.168 Kb (3056 amino acids) [Savitsky et al., 1995]. The entire coding region (66 exons) and splicing sites of the A–T gene were amplified from genomic DNA and examined for mutations by direct DNA sequencing or heteroduplex analysis. The clinical phenotype, as measured by survival and growth of A–T patients, was correlated with different mutation types.

MATERIALS AND METHODS

Subjects

A–T families in the United States and Canada were studied with informed consent from each study participant under a protocol approved by the New York Medical College Committee for the Protection of the Rights of Human Subjects. The diagnosis of A–T in the propositi was confirmed from clinical records as described previously [Swift et al., 1991]. For each A–T patient in the study, health information, medical data, vital status, date of birth, and of death when applicable, were

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obtained through study questionnaires, medical records and death certificates.

Mutation Detection

Genomic DNA was isolated from peripheral blood or paraffin-embedded tissue using standard procedures [Sambrook et al., 1989].

The A–T locus was screened for mutations by amplifying each exon of the A–T gene and its splicing sites from genomic DNA obtained from A–T index cases or parents. The polymerase chain reaction (PCR) was performed in 25 μl reaction volumes using a GeneAmp PCR System 9600 (PE Applied Biosystems, Foster City, CA). The PCR mixture contained 50 ng DNA, 5 mmol/l of each dNTP (Boehringer Mannheim, Indianapolis, IN), 1 unit of AmpliTaq Gold DNA Polymerase (PE Applied Biosystems, Foster City, CA) and 5 pmol of each sense and anti-sense primer (primers information available upon request) in a reaction buffer (1.5 mmol $MgCl_2$, 10 mmol Tris-HCl pH 8.3, 50 mmol KCl). The reaction was first heated at 95°C for 10 min, followed by 30 cycles of PCR amplifications (95°C 30 sec, 60°C 30 sec and 72°C 30 sec).

Initially, heteroduplex analysis on Mutation Detection Enhancement gels (FMC Bioproducts, ME) was used to detect mutations in DNA from more than 52 obligate heterozygotes. Each exon in which a heteroduplex was detected was sequenced. The PCR products were directly sequenced by the Big Dye Primer Cycle Sequencing Reactions using the ABI 877 Integrated Thermal Cycler and ABI 377 Automated Sequencer (Perkin-Elmer Applied Biosystems, CA). Sequence variations detected in this way were evaluated as described below.

Exon-by-exon PCR products from the remaining 90 A–T chromosomes (37 homozygotes and 16 obligate heterozygotes) were directly sequenced without preliminary screening. Mutations were sought in DNA from obligate heterozygotes when the homozygous probands DNA was not available.

DNA sequences from A–T chromosomes were compared with A–T gene sequence from GenBank (Accession number: U33841). Mutations in the coding regions were classified as missense, nonsense, frame shift, inframe deletion according to the predicted protein structure change in the gene product. Intragenic variations causing splicing errors were classified as splicing site mutation. Those sequence changes that would not result in a truncation or an amino acid change in the gene product were identified as neutral variants. These variants were excluded from remaining analyses.

Survival Analysis

For phenotype analysis the A–T patients were grouped as follows:

1. Missense group. All patients with one missense mutation.
2. Splice site group. All patients who had a single splice site mutation.
3. 2546Δ3 group. Each patient had this in-frame deletion on one chromosome.
4. Heterozygous for two truncations group. Patients had two different truncating mutations.
5. Homozygous truncation group. Nine of the ten mutations in this group truncated the A–T gene product before codon 1125.
6. Heterozygous for one truncation group. One truncating mutation on one chromosome 11, and another, unknown, mutation on the paired chromosome.

There was no overlap between the groups listed above. Two patients in the same family with one missense (R3008C) and one splicing (IVS59+1Δ4) mutation were excluded from the analysis. The cumulative survival of patients by mutation types was calculated by the Kaplan–Meier survival analysis and the mean survival of patients was compared using the Log Rank Test (significance at 5% level).

Height Centiles

The height of each patient was obtained from questionnaires, medical records, and telephone inquiries to the mothers of propositi. Age and gender-specific height centiles of A–T patients were measured using the Growth Chart from the National Center for Health Statistics [Hamill et al., 1976].

RESULTS

The Spectrum of A–T Mutations

Direct sequencing, without pre-screening, of the entire coding region of the A–T gene detected 77 mutations (85%) in 90 A–T chromosomes from 37 A–T homozygotes and 16 obligate heterozygotes. Previously, 42 mutations were found by heteroduplex analysis, and confirmed by sequencing. Of the 119 mutations detected, 71 were unique (Table I). One or both mutations were identified for 134 patients in 108 A–T families.

Most of the unique mutations (50/71, 70%) were found in only a single family. A–T patients from seven consanguineous families were homozygous for a single mutation. Four Amish patients were homozygous for 521ΔAG, and two A–T patients of Irish ancestry were heterozygous for this mutation. All chromosomes bearing this mutation shared the same microsatellite marker haplotype on chromosome band 11q22 (data not shown). An A insertion at codon 1904 was shared between 6 families and a 9 nucleotide in-frame deletion at codon 2546 between 8 families. The families with 1904insA and 2546Δ3 originated throughout Europe (French, British, German, Sweden, Dutch, and Polish).

Truncating mutations, frame shift and nonsense, were distributed throughout the A–T gene (Table II). Four of 9 (44%) missense mutations were found in codons 2401 ~ 3056, that is the COOH-terminal of the A–T gene product. The single in-frame deletion, 2546 3, and the three splicing-site mutations occurred at the C-terminal.

In addition, nine common neutral sequence variants, with a frequency over 5% in both A–T and non-A–T chromosomes, were identified in both intragenic and coding regions of the A–T gene [Li et al., 1999]. Nine rare variants were detected, each on a single A–T chro-

TABLE I. 71 Unique A–T Mutations* Detected in 119 A–T chromosomes

Unique mutation type (n = 71)	Exon	Codon	Nucleotide	Sequence change	Predicted protein change	Number of A–T chromosome (of homozygotes)[a]
Frame shift n = 46 (65%)	6	72	216	Del AG		1
	7	123	368	Del A		1
	7	124	370	Del 5		1
	9	264	790	Del T		1
	10	342	1027	Del 4		1
	10	352	1055	Ins T		3 (1)
	11	380	1139	Ins 4		2
	12	429	1287	Del TG		1
	12	468	1403	Del AA[b]		2
	12	521	1561	Del AG[b]		10 (4)
	13	585	1753	Del 4		1
	15	688	2062	Del G		1
	19	872	2614	Ins A		1
	20	897	2689	Del 5		1
	20	907	2720	Del 4		1
	24	1082	3245	ATC-TGAT[b]		3
	24	1092	3275	Ins A		1
	27	1209	3626	Del TT		2
	27	1238	3712	Del 5		1
	28	1268	3802	Del G[b]		1
	29	1368	4102	Del T		1
	31	1458	4372	Del G		1
	32	1508	4524	Ins A		1
	33	1546	4638	Del 4		1
	33	1581	4742	Ins A		1
	38	1775	5324	Del T		1
	39	1850	5549	Del T		1
	39	1852	5555	Ins CA		1
	40	1904	5712	Ins A[b]		6
	43	1978	5935	Del T		1
	43	2005	6015	Ins C[b]		1
	45	2076	6228	Del T		1
	46	2124	6371	Ins G		1
	50	2333	6997	Ins A[b]		3
	50	2337	7010	Del GT[b]		1
	52	2526	7578	Ins 367		2 (1)
	54	2544	7630	Del 11[b]		1
	54	2569	7705	Del GA		1
	56	2663	7989	Del 3		1
	59	2799	8395	Del 10		1
	60	2811	8432	Del A		1
	61	2886	8656	Ins T		1
	63	2934	8802	Del C		1
	63	2945	8833	Del CT[b]		1
	65	3001	9003	TTT-C		1
	65	3049	9145	Del TT		1
Nonsense n = 12 (17%)	5	57	170	G-A	W57X	2
	6	100	299	T-A	L100X	1
	8	199	597	T-A	C199X	1
	15	644	1931	C-A	S644X	1
	25	1124	3372	C-G	Y1124X	2 (1)
	37	1765	5293	C-T	Q1765X	1
	41	1924	5771	C-A	S1924X	1
	42	1978	5932	G-T	E1978X[b]	2
	52	2443	7327	C-T	R2443X[b]	2
	58	2819	8455	A-T	K2819X	1
	64	2960	8879	G-A	W2960X	1
	64	2993	8977	C-T	R2993X[b]	2
In-frame deletion n = 1 (1%)	54	2546	7638	Del 9	Del 3 aa[b]	8
Missense n = 9 (13%)	9	224	670	A-G	K224E	1
	10	323	967	A-G	I323V	1
	31	1420	4258	C-T	L1420P	1
	43	2032	6095	G-A	R2032K[b]	3
	48	2227	6679	C-T	R2218C	1
	55	2625	7875	TG-GC	D2625Q[b]	2
	60	2832	8494	C-T	R2832C	3
	60	2855	8565	TG-AA	S2855R, V2856I	1
	65	3008	9022	C-T	R3008C[b]	1

TABLE I. (Continued)

Unique mutation type (n = 71)	Exon	Codon	Nucleotide	Sequence change	Predicted protein change	Number of A–T chromosome (of homozygotes)[a]
Splicing site n = 3 (4%)	IVS54-2 A–C[b]			Del 159	2544Del 53 aa	5
	IVS59+1 del 4[b]			Del 150	2757Del 50 aa	4
	IVS62+1 G–A[b]			Del 115 or ins14	2891 or 2930 truncation	2

*Mutations were named according to Ad Hoc Committee on Mutation Nomenclature [1996]. All mutations were in compound heterozygotes except those labeled as "homozygote."
[a]Fifty mutations were private to single family.
[b]Previously reported. Fifty-one were novel mutations.

mosome. Two coding variants, D1853V (5558A>T) and T2438I (7313C>T), could be distinguished from a missense mutation, and four other intronic variants, IVS20-22ΔAAT, IVS26-4ΔT, IVS26-16ΔT and IVS56+75A>T, could be distinguished from splicing site mutations, because a truncating mutation was detected on the same A–T chromosome. Three single base substitutions, 2502A>G (E834E), 4272GT(D1424D) and 5793TC(A1931A), were considered to be neutral base changes because they did not alter encoded amino acids.

Survival of A–T Patients With Different Mutation Types

The survival times, mean survival and mutation types for the 134 A–T patients in the 6 groups defined in the Methods are described in Tables III and IV. The longest survival was observed in 18 patients with one missense mutation (33.3 ± 2.8 years), 12 patients with one splice site mutation (30.6 ± 2.7), or 10 patients with two heterozygous truncating mutations (32.1 ± 2.8). Two patients in the same family with one missense (R3008C) and one splicing (IVS59+1Δ4) mutation lived to ages of 38 and 45 years. The 12 A–T patients with the in-frame deletion, 2546Δ3, had a mean survival of 19.2 ± 2.2 years, and the 10 patients with two homozygous truncating mutations, had a mean survival of 21.8 ± 2.3 years, significantly lower than that of all other patients. The only patient in this group with a homozygous truncation at residue 2526 lived to age 32 years.

The median year of birth for the 12 A–T patients with the in-frame deletion, 2546Δ3, was 1961.5, for the patients homozygous for a truncation 1960, for the patients with a splice site mutation 1965, and for those with a missense mutation 1967. There were 12 families with multiple affected sibs in which the mutations were known. Two of these had only young living probands. Of the remaining 10, seven showed concordance between sibs in length of survival (Table III).

Among the remaining 72 A–T patients (Table IV) heterozygous for one truncating mutation (and one unknown), patients with truncations at or above residue 1800 lived longer than those with truncations before this residue. The mean survival of 25.9 years for patients with one truncating mutation within A–T residues 1 ~ 1800 was not significantly different, however, from that of patients with one truncating mutation within residues 1801 ~ 3056, 29.0 years.

Height of A–T Patients With Different Mutation Types

Height information and mutation types were available for 59 patients (Table V). The height of ten of 12 (83%) patients with 2546Δ3 and of all nine patients (100%) with homozygous truncating mutations was lower than the fifth centile. In contrast, two of nine (22%) with two different truncating mutations, eight of 12 (67%) with one splicing and eight of 17 (47%) patients with one missense mutation had heights greater than the fifth centile. The mean height of all patients with either 2546Δ3 or a homozygous truncation was significantly smaller than the mean height of all patients with either one missense, one splicing site, or two different truncating mutations (χ^2 test, two-sided $P = 0.0004$).

DISCUSSION

Reliable and sensitive mutation detection at the A–T locus is important because such mutations predispose carriers of a single A–T mutation to cancer and ischemic heart disease, and cause the autosomal recessive syndrome, ataxia-telangiectasia, in individuals who have an A–T mutation on both chromosome 11. Direct sequencing of all exons and splice junctions ("the gold standard") detected mutations in 85% of chromosomes

TABLE II. Locations of Unique A–T Mutations

Mutation types	A–T residues 0≈600	601~1200	1201~1800	1801~2400	2401~3056	Total
Missense	2		1	2	4	9
Nonsense	3	2	1	2	4	12
Frame shift	11	6	9	9	11	46
In-frame deletion					1	1
Splicing					3	3
Total	16	8	11	13	23	71

TABLE III. Survival of A–T Patients With Different Mutation Types*

Mutation	Family index	Mutation 1	Mutation 2[b]	Age (years) indicated[c]
One 2546Δ3	D1	2546Δ3	521Δ2	D5, D19, D31
(n = 12)	D2	2546Δ3	W57X	D11, D11
	D3	2546Δ3	ND	L18, L18
	D4	2546Δ3	ND	D20
	D5	2546Δ3	ND	D20
	D6	2546Δ3	ND	D21
	D7	2546Δ3	ND	D23
	D8	2546Δ3	ND	D24
Homozygous for a truncation	THm1	352insT	352insT	D10
(n = 10)	THm2	521Δ2	521Δ2	D16, D19
	THm3	521Δ2	521Δ2	D20
	THm4	521Δ2	521Δ2	D19, D21, L28
	THm5	521Δ2	521Δ2	D30
	THm6	Y1124X	Y1124X	D20
	THm7	2526ins 367	2526 ins 367	D32
Heterozygous for two different truncations	THt1	W57X	3001TTT-C	L11
(n = 10)	THt2	907Δ4	2333ins A	D13
	THt3	1508ins A	1904ins A	L16
	THt4	1546Δ4	E1978X	L18
	THt5	1092ins A	R2443X	L19
	THt6	123ΔA	1904ins A	L24
	THt7	380ins A	K2819X	L27
	THt8	264ΔT	3049Δ2	L31
	THt9	872ins A	1904 ins A	D34
	THt10	585Δ4	1209Δ2	D35
One splicing[a]	S1	IVS54-2A-C	1581ins A	L14
(n = 12)	S2	IVS54-2A-C	ND	D17
	S3	IVS54-2A-C	2886insT	D30
	S4	IVS54-2A-C	ND	L33
	S5	IVS54-2A-C	S644X	D35
	S6	IVS59+1Δ4	ND	D17
	S7	IVS59+1Δ4	430Δ2	D20
	S8	IVS59+1Δ4	ND	D29, D37
	S9	IVS62+1G-A	ND	L33, L36
	S10	IVS62+1G-A	ND	D39
One missense[a]	M1	R2032K	ND	L6, L10
(n = 18)	M2	D2625Q	S1924X	L12
	M3	R2832C	ND	L14, L17
	M4	R2218C	ND	D18
	M5	I323C	ND	D19
	M6	K224E	688ΔG	D20
	M7	S2855R, V2856I	ND	L21
	M8	R2032K	ND	L30, L33
	M9	L1420P	ND	L31
	M10	S2855R, V2856I	ND	D36
	M11	R2832C	ND	D36, D38
	M12	R2832C	ND	D20, L39
	M13	R2032K	ND	L44

*The groups are defined in the methods.
[a]Two patients, in the same family, died at age 38 and 45 years. They had one missense (R3008C) and one splicing site mutation (IVS59+1Δ4) on their A–T chromosomes and were not included in the analysis.
[b]ND = not detected.
[c]Patients vital status: L, living; D, died.

known to cause A–T in homozygotes. The remaining 15% are likely to be found in interior portions of introns.

Mutations of different types and sites led to different clinical phenotypes. Poor growth has been observed in many A–T patients [Boder and Sedgwick, 1958]. Fibroblasts from A–T patients have been shown to grow more slowly in culture [Elmore and Swift, 1976]. A–T patients also have a markedly shortened lifespan, with a chronic progressive pulmonary disorder as the principal cause of death [Morrell et al., 1986]. Height and length of survival are phenotypic characteristics that can be compared quantitatively. In contrast, it is difficult to measure precisely the degree of severity of the important neurological manifestations of A–T, although a general impression of mildness or severity can be formed. Although quantifiable data could not be obtained, it was the general impression that neurological symptoms and progression were milder in A–T patients who survived until their 30s.

Frameshift (insertions and deletions) and nonsense mutations lead to truncation of the A–T gene product. Such truncated products seem to have different clinical effects depending on the site of truncation, because the

TABLE IV. Mean Survival of A–T Patients With Different Mutation Types*

Mutation type	Number of patients	Mean ± SE (years)	95% Confidence interval	Log Rank Test (significance at 5% level)
One 2546Δ3	12	19.2 ± 2.2	14.9 ~ 23.4	vs. one missense ($P = 0.001$) vs. one splicing ($P = 0.005$) vs. two hetero-truncation ($P = 0.002$) vs. one hetero-truncation ($P = 0.03$)
One missense	18	33.3 ± 2.8	27.8 ~ 38.9	
One splicing	12	30.6 ± 2.7	25.3 ~ 35.9	
Heterozygous for two different truncations	10	32.1 ± 2.8	26.7 ~ 37.6	
Homozygous for a single truncation	10	21.8 ± 2.3	17.4 ~ 26.2	vs. one missense ($P = 0.004$) vs. one splicing (1) ($P = 0.02$) vs. two hetero-truncation ($P = 0.008$)
Heterozygous for one truncation; the other mutation unknown	72	28.4 ± 1.8	24.8 ~ 32.0	

*The groups are defined in the Methods.

group of patients with truncations before residue 1125 had significantly worse survival and growth those whose truncations were above that cut-off point. For truncation it could be that the length of the gene product determines the severity of the clinical phenotype.

In addition, patients with one missense or one splice site mutation were taller and had significantly longer survival than patients with severely truncated gene products. Missense mutations change a single amino acid in the A–T gene product and lead to an alteration, but not abolition, of function. For splice site mutations, it is possible that alternative splicing produces some normal gene product, that might account for the milder phenotype [McConville et al., 1996].

Not all mutations near the C-terminal, however, had mild clinical effects. The in-frame deletion 2546Δ3 was also associated with poor survival. This is the only known in-frame deletion at the A–T locus. It was observed frequently in our series (8 families) and previous reports (12 families) [Byrd et al., 1996; Gilad et al., 1996a, b; Wright et al., 1996; Watters et al., 1997; Telatar et al., 1998]. This deletion, occurred upstream from the PI3-kinase domain of the gene product, has been shown to produce an almost full-length product [Watters et al., 1997]. Is there something about the gene product from this deletion that leads to function even worse than that associated with the virtual absence of the gene product? The reasons for this deletion's severe survival and growth effects and its high frequency among A–T patients (7%) need to be investigated.

The differences between the different groups in length of survival cannot be attributed to the approximately six year difference in median year of birth between the longest surviving and shortest surviving groups of patients, because medical care for, and survival of, A–T patients never advanced dramatically over any six year interval.

Previously, a "milder clinical phenotype" was noted for two patients in one family with the single missense mutations 7271T→G, and survival past age 60 was reported for a patient homozygous for this mutation [Stankovic et al., 1998]. No other A–T patient with missense mutations on both paired chromosomes has been found.

According to previous reports, two of the splice site mutations in our series were associated with skipping of a single exon [Wright et al., 1996; Stankovic et al., 1998; Telatar et al., 1998], and the third, IVS62+1G–A, resulted in two different splicing defects, 2891Δ115 and 2930ins14 [Wright et al., 1996; Stankovic et al., 1998]. Survival data were not given for previously reported patients with these specific mutations. On the other hand, another splicing site mutation, IVS35+5A–G, led to a 137 bp insertion and a "milder clinical phenotype" [McConville et al., 1996].

These observations raise the possibility that some A–T families or physicians will request mutation analysis for their patients for prognostic purposes. Additional data from other laboratories is urgently needed to determine whether the correlation we observed is also seen in other settings. If further data confirm what we have found, mutation analysis may help estimate the prognosis of A–T patients.

The number of unique mutations detected in A–T patients continues to grow steadily as mutations are detected in previously unstudied A–T chromosomes. In the present series, only 20 of the 71 unique mutations detected had been previously reported among the approximately 200 A–T chromosomes examined by a variety of methods [http://www.vmmc.org/vmrc/atm.htm]. The distributions of mutation types (Table I) and locations (Table II) in the present study are representative of all mutations, in the coding region and splice sites, that can lead to clinical A–T.

Frameshift (insertions and deletions) and nonsense mutations were distributed throughout the gene. An excess of single base substitutions at both gene terminals may be due to a structural predisposition to point mutations or the functional significance of these two regions [Khanna et al., 1998]. All three splice site mutations in this study, and the in-frame deletion 2546Δ3, were near the C-terminal end of the gene. Splice site mutations elsewhere in the gene, however, have been

TABLE V. Age and Sex-Specific Heights Percentiles of 59 A–T Patients With Different Mutation Types*

	Percentile (%)						
Mutation	<5	5	10	25	50	75	Total
One 2546Δ3	10	1	1				12
							9
Homozygous for a truncation	9						
Heterozygous for two different truncations	7			1	1		9
One splicing	4	2	1		4	1	12
One missense	9	1	2	4		1	17

*The groups are defined in the Methods.

reported previously [http://www.vmmc.org/vmrc/atm.htm]. The C-terminal of the gene product corresponds to the phosphatidyl-inositol 3 (PI3)-kinase domain and is involved in cellular signal transduction and DNA damage responses [Savitsky et al., 1995; Khanna et al., 1998].

Methods based on exon-by-exon screening of PCR products from genomic DNA have two advantages. First, genomic DNA can be easily obtained from peripheral blood, saliva, or fixed tissue samples. Second, it detects a higher proportion of mutations on chromosomes known to carry an A–T mutation than does the Protein Truncation Test or SSCP of mRNA [Sasaki et al., 1998; Stankovic et al., 1998]. Cheaper, faster methods based on PCR products from genomic DNA, such as heteroduplex analysis on chromatography columns, should be investigated to determine if they are as sensitive in detecting mutations as direct sequencing [Transgenomic Wave System, www.transgenomic.com].

There are also limitations to this approach. It usually will not detect mutations deep within introns that lead to altered splice sites. Further. it detects both functional and neutral alterations in DNA sequence. Many non-functional sequence alterations can be identified as neutral variants because they have already been characterized as such [Li et al., 1999]. cDNA analysis may help evaluate the functional significance of sequence variations within splice sites and be necessary to find mutations, in A–T chromosomes, that are not detected by exon-by-exon sequencing.

The distribution of mutations at the A–T locus in population samples unrelated to A–T families cannot be predicted. It is possible, for example, that some missense or splice site mutations, particularly in the interior of the gene, affect function but do not lead to clinical A–T when paired with another mutation at this locus. Such mutations might be detected in screening the general population or groups of cancer patients.

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REFERENCES

Ad Hoc Committee on Mutation Nomenclature. 1996. Update on nomenclature for human gene mutations. Hum Mut 8:197–202.

Athma P, Rappaport R, Swift M.1996. Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92:130–134.

Boder E, Sedgwick RP. 1958. Ataxia-telangiectasia. A familial syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and frequent pulmonary infection. Pediatrics 21:526–554.

Byrd PJ, McConville CM, Cooper P, Parkhill J, Stankovic T, McGuire GM, Thick JA.1996. Mutations revealed by sequencing the 5′ half of the gene for ataxia telangiectasia. Hum Mol Genet 5:145–149.

Elmore E, Swift M.1976. Growth of cultured cells from patients with ataxia-telangiectasia. J Cell Physiol 89:429–431.

Gilad S, Bar-Shira A, Harnik R, Shkedy D, Ziv Y, Khosravi R, Brown K, Vanagaite L, Xu G, Frydman M, Lavin MF, Hill D, Tagle DA, Shiloh Y.1996a. Ataxia-telangiectasia: founder effect among North African Jews. Hum Mol Genet 5:2033–2037.

Gilad S, Khosravi R, Shkedy D, Uziel T, Ziv Y, Savitsky K, Rotman G, Smith S, Chessa L, Jorgensen TJ, Harnik R, Frydman M, Sanal O, Portnoi S, Goldwicz Z, Jaspers NGJ, Gatti RA, Lenoir G, Lavin MF, Tatsumi K, Wegner RD, Shiloh Y, Bar-Shira A. 1996b. Predominance of null mutations in ataxia-telangiectasia. Hum Mol Genet 5: 433–439.

Hamill PVV, Drizd TA, Johnson CL, Reed RA, Roche AF.1976. NCHS Growth Chart. Monthly Vital Statistical Report: Health Examination Survey Data from the National Center for Health Statistics. Washington DC: US Department of Health, Education and Welfare.

Khanna KK, Keating KE, Kozlov S, Scott S, Gatei M, Hobson K, Taya Y, Gabrielli B, Chan D, Lees-Miller SP, Lavin MF.1998. ATM associates with and phosphorylates p53: mapping the region of interaction. Nat Genet 20:398–400.

Li A, Huang Y, Swift M. 1999. Neutral sequence variants and haplotypes at the 150 Kb ataxia-telangiectasia locus. Am J Med Genet 86:140–144.

McConville CM, Stankovic T, Byrd PJ, McGuire GM, Yao QY, Lennox GG, Taylor MR. 1996. Mutations associated with variant phenotypes in ataxia-telangiectasia. Am J Hum Genet 59:320–330.

Morrell D, Cromartie E, Swift M. 1986. Mortality and cancer incidence in 263 patients with ataxia-telangiectasia. J Nat Cancer Inst 77:89–92.

Sambrook J, Fritsch EF, Maniatis T.1989. Molecular cloning: a laboratory manual, 2nd ed. New York: Cold Spring Harbour Laboratory Press. p 25–27.

Sasaki T, Tian H, Kukita Y, Inazuka M, Tahira T, Imai T, Yamauchi M, Saito T, Hori T, Hashimoto-Tamaoki T, Komatsu K, Nikaido O, Hayashi K. 1998. ATM mutations in patients with ataxia-telangiectasia screened by a hierarchical strategy. Hum Mut 12:186–195.

Savitsky K, Bar-Shira A, Gilad S, Rotman G. Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Sankhavaram RP, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NGJ, Taylor AMR, Arlett CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y. 1995. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749–1753.

Stankovic T, Kidd AMJ, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T, Bradwell AR, Easton DF, Lennox GG, Haites N, Byrd PJ, Taylor AMR.1998. ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma and breast cancer. Am J Hum Genet 62:334–345.

Swift M, Morrell D, Massey RB, Chase CL.1991. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 325:1831–1836.

Telatar M, Teraoka S, Wang Z, Chun HH, Liang T, Castellvi-Bel S, Udar N, Borresen-Dale A, Chessa L, Bernatowska-Matuszkiewicz E, Porras O, Watanabe M, Junker A, Concannon P, Gatti RA.1998. Ataxia-telangiectasia: identification and detection of founder mutations in the ATM gene in ethnic populations. Am J Hum Genet 62:86–97.

Watters D, Khanna KK, Beamish H, Birrell G, Spring K, Kedar P, Gatei M, Stenzel D, Hobson K, Kozlov S, Zhang N, Farrell A, Ramsay J, Gatti R, Lavin M. 1997. Cellular localization of the ataxia-telangiectasia (ATM) gene product and discrimination between mutated and normal forms. Oncogene 14:1911–1921.

Wright J, Teraoka S, Onengut S, Tolun A, Catti RA, Ochs HD, Concannon P. 1996. A high frequency of distinct ATM gene mutations in ataxia-telangiectasia. Am J Hum Genet 59:839–846.